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TREATMENT OF EXPERIMENTAL TUBERCULOSIS  
WITH ANTITOXIC SERUM.

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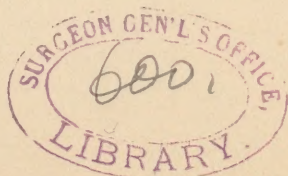
## SOME PRODUCTS OF THE TUBERCULOSIS BACILLUS AND THE TREATMENT OF EXPERIMENTAL TUBERCULOSIS WITH ANTITOXIC SERUM.

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So much has been written in regard to the poisons of the tuberculosis bacillus that a review on this occasion would demand too much time, and we desire to refer only briefly to the work which is of importance in connection with those substances which we will describe.

Tuberculin, as is well known, is the extract of the tuberculosis bacilli, including the media upon which they are grown. From specially prepared artificial cultures of the tuberculosis germ Kühne<sup>1</sup> and the writer<sup>2</sup> (*Bulletin No. 7, Bureau of Animal Industry*) have obtained a substance corresponding to a nucleo-albumin which appeared to be the fever-producing principle of the germ. However, many conditions in tuberculosis were not accounted for by this substance, and as Mafucci,<sup>3</sup> Prudden and Hodenpyl,<sup>4</sup> Vissman,<sup>5</sup> and others had succeeded in producing tubercular nodules without necrosis by the intravenous injection of dead bacilli, it seemed as though it should be possible to isolate either from cultures or from bodies of the germs themselves some substance which might be considered accountable for the coagulation-necrosis of tissue which takes place—a necrosis which it appears is necessary for the progress of the disease. This problem was undertaken with the co-operation of my assistant, Dr. Dorset, more than two years ago. After many fruitless attempts we succeeded in isolating from artificial liquid cultures a crystalline substance having a melting-point of 161° to



164° C., readily soluble in ether, alcohol, and water, which separated from these solutions in needle-like or prismatic crystals (Plate I.) showing a slight yellow tint. They did not give the biuret reaction. The solution of this substance has an acid reaction to litmus, is acid in taste, and is optically inactive. The crystals give no precipitate with silver nitrate ( $\text{Ag}.\text{NO}_3$ ), platinum chloride ( $\text{PtCl}_4$ ), or barium hydrate ( $\text{Ba}(\text{OH})_2$ ). The analysis showed C = 50.88 per cent.; H = 6.70 per cent.; O = 42.42 per cent., giving a formula corresponding closely to  $\text{C}_7\text{H}_{10}\text{O}_4$ . This is the formula of teraconic acid, an unsaturated acid of the fatty series.

The culture-media upon which the germs were grown and from which these crystals were obtained contained potassium acid phosphate, ammonium phosphate, asparagin, and glycerine—the media used and described by one of us (de Schweinitz<sup>6</sup>) some years ago for studying bacterial products. After the germ has been growing on this media for some weeks the liquid becomes light yellow in color, having the appearance of a pale urine—a change which does not take place in the uninoculated media kept under the same conditions. Efforts to obtain this same acid from the ordinary beef-broth cultures containing peptone and glycerine resulted in securing minute amounts of the crystals only, which it was never possible to purify. After noting some of the other properties of this acid substance we came to the conclusion that the presence of peptone and the nitrogenous bases of the meat resulted in their combination with the crystals, forming compounds from which the acid could not again be easily extracted, even after the addition of acid. Finally, a small quantity of the crystalline substance obtained from the artificial cultures was added to the glycerine peptonized beef-broth media, but it was impossible to recover it again by the methods used for the first extraction, viz., repeated precipitation with alcohol and extraction with water and ether. The ready solubility of this substance in water as well as ether probably accounts for the difficulty of obtaining it. The uninoculated media did not yield these crystals. When dissolved in water and injected into guinea-pigs the following effects were noted. The injections were made subcutaneously in the thigh :

- I. Healthy guinea-pigs: No. 314 received 0.015 gramme of crystals.  
Temperature at time of injection 102.6° F.  
Temperature, 11.50, one hour after injection, 100.6° F.



PLATE I.





Temperature, 1.30 P.M., 100.2° F.

Temperature, 3.00 P.M., 102.4° F.

Temperature, 4.00 P.M., 102.2° F.

During the above period breathing was rapid, with an occasional rigor.

Guinea pig No. 422. Weight 284 grammes; received 0.0095 gramme of crystals at 12.05 P.M.

Temperature at time of injection 99.8° F.

Temperature at 2.30 P.M. 97.4° F.

On the next day there was quite perceptible swelling where the injection was made. Pig was chloroformed at end of twenty-four hours and showed considerable inflammation at seat of injection. Tissues were hemorrhagic and bathed in a serous exudate. The muscular tissue was much disintegrated, resembling the appearance from the action of a caustic.

Guinea-pig No. 511. Weight 183 grammes; received 0.0048 gramme in  $\frac{1}{2}$  c.c. water at 11.25 A.M., subcutaneously in thigh.

Temperature at time of injection 103° F.

Temperature at 12.25 P.M., one hour after, 101.8° F.

Temperature at 1.15 P.M. 102° F.

Temperature at 3.25 P.M. 100.8° F.

Chloroformed next day. Considerable inflammation, with serous exudate at seat of injection.

Guinea-pig No. 10 received 0.0274 gramme at 10.10 A.M.

Temperature at time of injection 99.2° F.

Temperature at 10.40 A.M. 100.2° F.

Temperature at 11.15 A.M. 100.6° F.

Temperature at 11.50 A.M. 100.6° F.

Temperature at 2.00 P.M. 100.2° F.

During above period this pig showed signs of restlessness, breathed rapidly and shivered.

## II. Tuberculous guinea-pigs:

Guinea-pig No. 181 received 0.017 gramme at 10.25 A.M.

Temperature at 11.40 A.M. 101.4° F.

Temperature at 1.50 P.M. 102.8° F.

Temperature at 2.50 P.M. 103.4° F.

Temperature at 3.50 P.M. 103° F.

Pig sat drawn up in cage, and shivered.

Guinea-pig No. 259 had received virulent tuberculosis two weeks previous to injection of crystals. Received 0.0172 gramme at 10.45 A.M.

Temperature at time of injection 102.4° F.

Temperature at 11.45 A.M. 101.6° F.

Temperature at 3.20 P.M. 101.6° F.

Distinct rigors and rapid breathing.

Guinea-pig No. 377 inoculated with sputum from tuberculous patient some time before injection of crystals. Received 0.023 gramme at 11.35 A.M.

Temperature at time of injection 101.2° F.

Temperature at 11.35 100.6°



Trembling very noticeable.

Guinea-pig No. 11 had been inoculated with attenuated and virulent tuberculosis culture (weight 448 grammes). Received 0.0096 gramme of crystals at 11.25 A.M.

Temperature at time of injection 103° F.

Temperature at 12.25 P.M. 100.8° F.

Temperature at 1.15 P.M. 101° F.

Temperature at 3.25 P.M. 100.8° F.

The idea was suggested from these experiments that this acid, evidently a secretory product of the bacillus, was one of its most powerful weapons; that by its action upon the tissue the cells were first destroyed, so that they could subsequently be utilized by the germ as food, and in this way the germ protected itself from surrounding leucocytes. To test this, crystals dissolved in sterile water were injected by means of a hypodermic syringe directly into the liver. At the same time an equal quantity of water was injected into a check in the same way. After forty-eight hours check and experimental animals were killed. The check failed to show any effect, while the others exhibited a liver with several light spots. A repetition of this experiment gave the same results.

No effort was made to recover these crystals from the liver, as the amount used was too small. We did not test the effect upon the liver by an intravenous injection, as would otherwise have been done, because we had found that there was a combination of this acid substance with the albuminoids or bases, and any intravenous injection would have resulted in its immediate conversion into a modification by uniting with the albuminoids in the blood. Further, the growth of the germ in the body is localized, and where localized the necrotic areas are apparent, so that the fairest test was to bring the substance as soon as possible in contact with the tissue. The experiments in injections of the animals and appearance of sections follow.

Injection of crystals from artificial cultures of *B. tuberculosis* into the liver of guinea-pigs:

Guinea-pig No. 409 received 0.00178 gramme in liver on left side at 11.45 A.M. Pig weighed 338 grammes.

Temperature at time of injection 101.6° F.

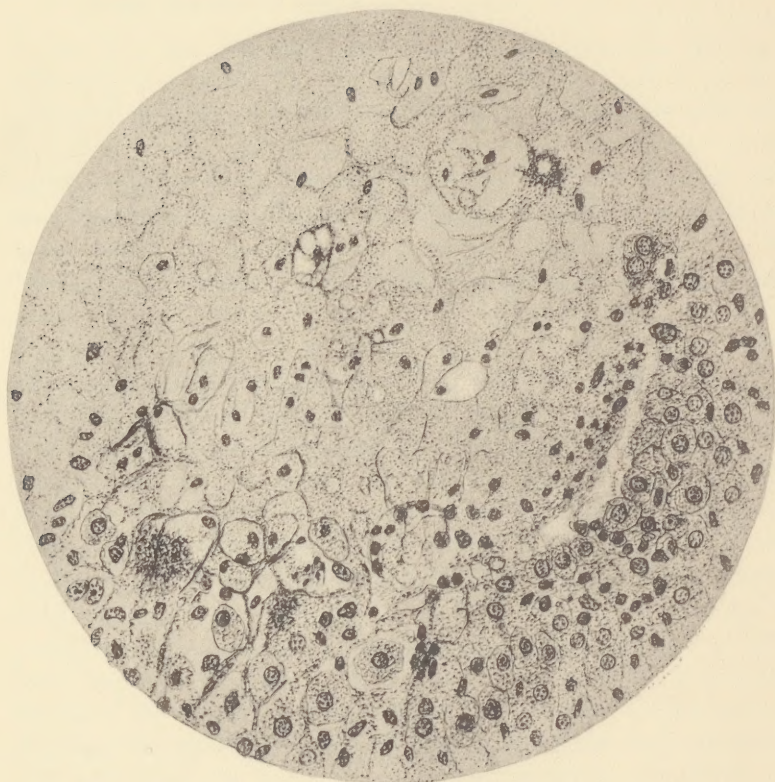
Temperature at 3.00 P.M. 102° F.

Chloroformed October 24, 1896, at 12 M. Liver dark, with one or two small





PLATE II.





white spots in it, and apparently a small inflamed spot at about the position where injection was made. Gall-bladder injected and seemingly inflamed.

Guinea-pig No. 412 received 0.0037 gramme in liver at 1.45 P.M. Pig weighed 242 grammes.

Temperature at 1.45 P.M. 103° F.

Temperature at 3.30 P.M. 100.4° F.

Chloroformed at end of forty-eight hours. Gall-bladder was congested (not so much as in No. 409). Liver showed pale spots and one or two small white areas of apparent necrosis. Hardened in  $\text{HgCl}_2$  on microscopic examination one area rather well defined where the liver-cells do not take hæmatoxylon well, though nuclei stain, could be seen.

Checks on injection of crystals into liver.

Guinea-pig No. 510 received  $\frac{1}{2}$  c.c. sterile distilled water in liver.

Chloroformed after forty-eight hours. Post-mortem, all organs, liver, lungs, spleen, etc., normal.

Guinea-pig No. 387 received  $\frac{1}{2}$  c.c. sterile distilled water in liver. Chloroformed after forty-eight hours. Post-mortem. All organs normal excepting one or two very small pale spots in liver; no necrosis.

Injection of crystals. Guinea-pig "C" received 0.0043 gramme in liver. Chloroformed after six days. Pig weighed 600 grammes.

Lungs very slightly congested. In large left lobe of liver there were two or three comparatively large areas of necrosis. These spots were on the side in which injection was made, and the liver appeared to show the track of needle. The guinea-pig was otherwise healthy.

Guinea-pig "E" received 0.0023 gramme of crystals in liver. Chloroformed after two days. Pig weighed 345 grammes.

All organs appeared normal, except stomach, which showed a slight inflammation in its wall on the side which lay next to a necrosed spot in the liver.

Besides this spot there were several others of considerable size in the liver on the side on which the injection was made. The section of pig "C," the one allowed to live six days after injection, showed on microscopic examination the following:

Stained with hæmatoxylon and eosin; distinct areas of necrosis were noted, the most marked ones near the surface of the liver. Polynuclear leucocytes were present, though not in large numbers, in and around the necrotic areas, and there was also an increase in connective-tissue cells of the liver around these same areas. Plate II. is a drawing of the liver section, showing healthy and necrosed areas.

Prudden,<sup>7</sup> 1892, suggests that caseation, so constantly present in tuberculosis, is probably due to a specific metabolic product of the bacillus.

It seems very reasonable to conclude from our experiments that we have here the substance formed by the bacillus which is responsible for this necrosis.

The formula which can be deduced from the analysis makes this acid correspond closely to teraconic, which has some properties very similar to those noted by us in connection with this new acid. Its identity we have not yet proved or disproved, as the preparation of teraconic acid is not completed. The amount of this acid obtained is very small, so that we have used only a very minute amount of it for testing its immunizing property. A single injection of 0.0020 gramme was sufficient to keep the animals alive some weeks longer than the checks, and its solution appeared to exert some slight bactericidal influence.

As this substance seemed to be a temperature-reducing principle in healthy and diseased animals, we endeavored to separate the fever-producing principle independently. The crystals were always found in the culture-liquid, and only minute amounts could be obtained from the germs themselves that had been grown on liquid media. Accordingly these bacilli, carefully filtered, without heat, were washed in cold water and next extracted with hot water. This hot-water extract contained an albuminoid which caused the tuberculin reaction in tuberculous guinea-pigs and calves upon repeated injections.

Roux and Nocard<sup>8</sup> claim that they have a tuberculin which will give reactions almost indefinitely, but do not describe its method of preparation. Whether this is the same substance that we have obtained we are unable to say, but certain it is that the tuberculin prepared in the way we have indicated will give reactions four to five times and more in succession, where the reaction with tuberculin as prepared in the ordinary way fails after the second time. The conclusion is a fair one. We think that the fever-reducing principle having been removed to an extent, if not entirely, the immunity to the fever-producing principle is much more slowly acquired. Our tests upon guinea-pigs and tuberculous calves were made with only one day intervening between the injections. See Table I.

In the *Deutsche med. Wochenschrift*, April 1, 1897, Dr. R. Koch<sup>9</sup> describes some new tuberculin preparations. The dried tuberculosis bacilli were taken (the culture media used is not mentioned), finely powdered and centrifugalized with distilled water. The opalescent solution obtained tested upon animals gave the tuberculin reaction.

The residual germs were submitted to this treatment a number of times until finally all were practically dissolved. The latter solutions in large doses caused a reaction, but in small quantities did not produce this result, and seemed to exert both an immunizing and curative action in experimental tuberculosis. Koch used for this work virulent germs, and claims that attenuated germs do not give an active product. Our own work was done with germs purposely attenuated by cultivation, and the results show that a very active fever-producing, fever-reducing, and probably curative principle can be obtained from them. It hardly seemed justifiable to ourselves or others to powder dried virulent germs and have the dust floating in the air. Koch further refers to two fatty acids, which, in conjunction with Proskauer,<sup>9</sup> had been found in the bodies of the germs. The writers<sup>10</sup> of this paper published in August, 1895, a preliminary study of the fats of the tuberculosis bacilli, showing the high content of fat in the body of these germs, which accounts for the difficulty in staining them with certain colors, as well as their difficult absorption.

In a later paper,<sup>11</sup> 1896, we described briefly the different acids obtained from the body of the germ—both high melting and low melting acids—but whether or not these are identical with those observed by Koch and Proskauer we cannot tell from the brief mention they have made of them.

From our results it seems very reasonable to think that the necrotic acid is the fever-reducing principle, the albuminoid the fever-producing principle, and the reason the ordinary tuberculin does not react continuously is on account of the presence of both of these substances at the same time. At any rate, tuberculous guinea-pigs tested successively with tuberculin showed no reaction, while with this albuminoid, which we will call cell-extract, a reaction was obtained.

The preliminary experiments published by one of us<sup>12</sup> in 1894 upon the production of an immunity or resistance to tuberculosis by attenuated cultures have been continued, and are confirmatory of the first results, showing the production of great resistance and in some cases complete immunity. A detail of two sets of these experiments may be given as an instance of their general results. (Table II.)



The first effect of the injection of the attenuated germ was in some instances to cause a slight decrease in weight, sometimes a local swelling was noted at the point of injection, and occasionally an enlargement of the inguinal glands. This disappeared after some weeks. This local swelling is probably due to the mechanical action of the bodies of the germs, on account of their high fat content and possible presence of a minute amount of the acid, causing necrosis. It does not always result from a subcutaneous inoculation, and an apparent immunity to this action is acquired by repeated injections. This is well shown in horses and cows submitted to treatment with the attenuated germ.

From six to eight weeks after the date of the injection of the germ guinea-pigs seem to be entirely well, and are then inoculated with the virulent germ. As can be seen from the chart, the checks died within six weeks from date of inoculation, while the others vaccinated remained well four months afterward. It has appeared from many experiments that if the inoculation with the virulent bacillus is made before complete recovery from the treatment with the attenuated bacillus the resistance is considerably less. The inoculation of the animals with the virulent bacillus, and subsequently with a single injection of the attenuated bacillus, showed that the latter produced a slight resistance, but no very material retardation of the disease.

The production of this partial immunity or artificial resistance by means of the attenuated cultures suggested already, in 1894, the availability of this same material for the purpose of treating animals for the production of a serum which would have some effect in curing tuberculosis. It suggested the idea further that possibly cattle could be vaccinated with this attenuated germ and made immune to tuberculosis.

Two cows and one heifer were selected for the work, which was conducted for us by Dr. Schroeder, in charge of the Experiment Station of the Bureau of Animal Industry. One of these animals was originally tuberculous, the other two healthy. The tuberculous animal received large doses of tuberculin until it had received altogether 19,407 c.c. (nineteen and one-half liters), and as much as 1500 c.c. of tuberculin at a single dose, from November, 1894, to April 20, 1897. The other animals received injections of the at-

tenuated culture, the amount injected in fifteen months being 11,425 c.c. and 18,100 c.c., respectively; and by this is meant the liquid culture-media *in toto*, including the germs, just as taken from the incubator, without any further treatment. At first the injections produced a slight reaction and occasionally a local œdema and abscess. After they had been continued for some time this effect diminished or disappeared. The serum of all of these animals was tested a number of times. Guinea-pigs were injected with the serum in quantities varying from  $1\frac{1}{2}$  to 6 c.c., and subsequently inoculated, together with the checks, with a culture sufficiently virulent to kill the checks within four or five weeks, or the pigs were inoculated with the virulent bacilli and treated by subsequent injections of the serum. Without giving the detail of the experiments, we may say that the serum from the cow treated with tuberculin would cause in the pigs a slight resistance to the disease; the serum of those treated with the attenuated bacilli produced more resistance on the part of the animals or prolonged their life to some extent, but not sufficiently, as compared with the quantity of material injected, to make the use of cow-serum appear practicable. The cow-serum, although sterile, frequently produced abscesses in guinea-pigs. This serum we expect to test again shortly, when it should be more potent.

While these experiments were in progress two horses had been pressed into service. They were treated by injecting the attenuated cultures, culture fluid, and bacilli. The first injection of 5 c.c. caused a decided temperature-reaction, local œdema, stiffness, slight loss of appetite, recovery after a few days. At first local abscesses were formed, which healed fairly readily. After a time the abscess-formation ceased or occurred occasionally. After eight months' treatment, the doses of the culture being gradually increased up to 300 to 400 c.c.—the total amount injected in fifteen months = 4459 c.c.—the serum was used for testing. It separated out clear and well. Two sets of illustrations may be given to show its action on tuberculous animals. (Table III.) In one set the checks and two treated pigs died; the other two treated pigs are alive and in perfect health, apparently, after a number of months. In another set the checks—four in number—died within four to five weeks, while the treated ones lived two or three weeks longer, showing, on autopsy, much

less disease in the lungs than the checks. We endeavored further to isolate from the serum antitoxic substances by a slight modification of the Brieger-Boer method. We finally succeeded in obtaining a small quantity of a grayish powder, giving the biuret reaction, soluble in water with difficulty, which was used for treating guinea-pigs in the same way as the serum. The result was as in the first instance. The pigs—one-half pound in weight—were inoculated with a virulent germ and treated by a single injection of 0.008 gramme of this solid substance. They lived three to four weeks longer than the checks, again showing considerable less disease in the lungs and less necrosis in the liver.

The effect of the serum was also tried in preventing the rise of temperature in tuberculous guinea-pigs and in saving them from a fatal dose of tuberculin. As can be seen from the temperature-reactions in Table I., the injections of  $\frac{1}{4}$  c.c. of diluted tuberculin and at the same time of  $\frac{1}{2}$  c.c. of the serum, either caused a decided reduction of the temperature or prevented a characteristic tuberculin reaction in animals weighing about 400 grammes.

The result of all this work leads us to the conclusion that the injection of the live culture in healthy animals produces substances antitoxic to tuberculosis; that the quantity of this substance can be increased gradually; that the treatment of tuberculosis is and will be for some time still in the experimental stage. One point, however, must be remembered, viz., that while it may be difficult to cure the disease in a guinea-pig, where its course is very rapid, virulent bacilli requiring only from four to five weeks to kill, it might be much easier to check the disease when more prolonged in action, as in the majority of cases in man. Again, in addition to some form of specific treatment for the disease, man usually has the advantage of being placed under the best possible surroundings as to diet, climate, etc., and every effort is made to aid the improvement of the patient, while with experimental animals the conditions are different. The experimental results obtained lead undoubtedly to the conclusion that while the treatment with antitoxic serum is still in the experimental stage, and should as yet be used only in sanitariums and under the best conditions, we are on the road to success in the treatment of this disease and nearer our goal than ever before.



In an experimental way, the antitoxic serum as prepared in my laboratory has been used by Dr. Stubbart at the Loomis Sanitarium, and some by Dr. Trudeau at Saranac Lake, as well as by Dr. Charles W. Richardson in this city.

The serum used for these cases was of the same lot that cured the guinea-pigs reported in Table III. The treatment has not been continued long enough for positive conclusions to be drawn. One of the cases reported by Dr. Stubbart has been apparently cured and the others much improved. Dr. Charles W. Richardson, of this city, reports decided improvement in the cases upon which this serum has been used. Dr. Trudeau has not used the material long enough to come to any definite conclusions. He noted reduction of high temperatures under its influence in several of the cases tried.

Maragliano, Babes, and Behring are the other principal workers in the preparation of an antitoxic serum for tuberculosis. Paquin also has an antitoxic serum on the market.

Maragliano<sup>13</sup> (*Revue de la Tuberculose*, Juillet, 1896, p. 131) gives the method he has used for the production of antitoxic serum, and notes that there is present in the cold filtered cultures of the tuberculosis bacilli a substance which causes the reduction of temperature, and another, not destroyed by heat, which causes the rise of temperature. In all probability, without isolating the principle, Maragliano was using solutions of the crystalline substance we have described in the beginning of this paper. While this is not destroyed by heat, as he seems to think, it does undergo some change by combining probably with the albuminoid matter in the media, and thus losing its distinct property as a temperature-reducing substance; or, more probably, its temperature-reducing property is disguised by the presence of the temperature-producing principle extracted by hot water. The serum which he obtains from treatment of the animals with the different products of the bacilli is claimed to have some effect in reducing the temperature and apparently improving the disease.

In the *Zeitschrift für Hygiene*, Babes,<sup>14</sup> reviewing a portion of the work upon the treatment of tuberculosis with serum, comes to the conclusion that he is the first individual to have discovered any antitoxic properties in this serum; that there is an antitoxic substance

present in treated animals, but that it has not yet been brought to a sufficient development to warrant general use.

Our experiments lead us to conclude that while the injection with tuberculin in healthy animals produces a serum containing antitoxic material, the amount of this is small, and that the injection of the live culture is the proper treatment. We cannot agree with the statement made that horses are unsuitable for the work. Mules and donkeys may perhaps give quicker results, but horses seem to be eminently satisfactory. At no time have we found that the horse-serum produces toxic effects, although this has been noted in the cow-serum.

If the antitoxic serum-treatment for tuberculosis could be freed for the present from its commercial aspect, and careful, systematic experiments continuously conducted in numerous hospitals and sanitariums, this or a similar modified method of treatment could be looked to for good results. When tuberculosis can be uniformly cured in guinea-pigs as certainly as diphtheria, then does the commercial aspect become a fair and legitimate one.

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TABLE I.—TEST OF CELL EXTRACT, TUBERCULIN, AND SERUM.

| Date.    | No. of Animal. | Condition.               | Weight in grams. | Substance injected.                                     | Temperature.   |                |               |
|----------|----------------|--------------------------|------------------|---------------------------------------------------------|----------------|----------------|---------------|
|          |                |                          |                  |                                                         | A.M.<br>11. 20 | P.M.<br>1. 40  | P.M.<br>3. 05 |
| March 26 | XI.            | Tuberculous Guinea-pig.  | 423              | $\frac{1}{4}$ cc. tuberculin + $\frac{1}{2}$ cc. serum. | 103. 2°        | 96. 6°         | 96. 2°        |
|          | 492            | Tuberculous Guinea-pig.  | 255              | $\frac{1}{4}$ cc. tuberculin.                           | 102. 8         | 105. 0         | 104. 4        |
|          | VIII.          | Attenuated, tuberculous. | 443              | $\frac{1}{4}$ cc. tuberculin + $\frac{1}{2}$ cc. serum. | 101. 0         | 102. 4         | 103. 8        |
|          | XIX.           | Attenuated, tuberculous. | 356              | 2 cc. cell extract = 0.0040 gram.                       | 102. 2         | 105. 6         | 103. 4        |
|          | 513            | Healthy (check)          | 210              | 1 cc. cell extract = 0.0020 gram.                       | 101. 4         | 101. 6         | 101. 0        |
| March 27 | XI.            | Tuberculous Guinea-pig.  | ...              | $\frac{1}{4}$ cc. tuberculin.                           | 101. 2°        | 102. 4°        | 102. 4°       |
|          | 492            | Tuberculous Guinea-pig.  | ...              | 2 cc. cell extract = $\frac{1}{4}$ cc. tuberc.          | 103. 0         | 103. 8         | 105. 0        |
|          | VIII.          | Attenuated, tuberculous. | ...              | $\frac{1}{4}$ cc. tuberculin.                           | 103. 0         | 103. 2         | 103. 8        |
|          | XIX.           | Attenuated, tuberculous. | ...              | $\frac{1}{4}$ cc. tuberculin.                           | 102. 8         | 103. 8         | 103. 0        |
|          | XX.            | Attenuated, tuberculous. | ...              | $\frac{1}{4}$ cc. tuberculin + $\frac{1}{2}$ cc. serum. | 102. 4         | 95. 0          | 95. 0         |
| March 29 | VIII.          | Attenuated, tuberculous. | ...              | 2 cc. cell extract = $\frac{1}{4}$ cc. tuberc.          | 101. 8°        | 103. 8°        | 103. 6°       |
|          | 492            | Tuberculous Guinea-pig.  | ...              | 2 cc. cell extract = $\frac{1}{4}$ cc. tuberc.          | 102. 8         | 104. 4         | 104. 6        |
|          | XIX.           | Attenuated, tuberculous. | ...              | 2 cc. cell extract = $\frac{1}{4}$ cc. tuberc.          | 102. 2         | 105. 6         | 103. 0        |
|          |                |                          |                  |                                                         | A.M.<br>10. 45 | P.M.<br>12. 15 | 2. 10<br>P.M. |
| March 30 | 492            | Tuberculous Guinea-pig.  | ...              | 2 cc. cell extract = $\frac{1}{4}$ cc. tuberc.          | 102. 2°        | 104. 2°        | 104. 2°       |
|          | XIX.           | Attenuated, tuberculous. | ...              | $\frac{1}{4}$ cc. tuberculin.                           | 102. 2         | 103. 2         | 103. 6        |



TABLE II.—TWO SETS OF EXPERIMENTS SHOWING THE AVERAGE RESULTS IN EXPERIMENTS IN WHICH THE GUINEA-PIGS WERE VACCINATED WITH ATTENUATED BACILLI AND THEN INOCULATED WITH VIRULENT BACILLI.

| No. | October 24. Inoculation and amount of attenuated germ. | Weight. |        | Condi- tion. | December 9. Inoculation and amount of virulent germ.   | Weight. |        |         |         |                           |
|-----|--------------------------------------------------------|---------|--------|--------------|--------------------------------------------------------|---------|--------|---------|---------|---------------------------|
|     |                                                        | Oct. 24 | Nov. 2 |              |                                                        | Dec. 16 | Jan. 7 | Jan. 19 | Feb. 2  | Feb. 8   Apr. 8   Apr. 19 |
| 373 | 1½ cc. 50th generation                                 | 12 oz.  | 14 oz. | O. K.        | Dead from pneumonia                                    | 14 oz.  | 16 oz. | 16 oz.  | 15½ oz. | 16 oz. 16 oz. 14 oz.      |
| 374 | " " "                                                  | 10 "    | 11 "   | "            | ⅛ cc. of vir. tuberculosis 4th generation from rabbit. |         |        |         |         |                           |
| 375 | " " "                                                  | 16 "    | 15 "   | "            | Dead from pneumonia                                    | 12 "    | 15 "   | 15 "    | 15 "    | 17½ " 18 "                |
| 376 | " " "                                                  | 16 "    | 14½ "  | "            | ⅓ cc. of vir. tuberculosis 4th generation from rabbit. | 12 "    | 13 "   | 12½ "   | Dead    |                           |
| 377 | Check .                                                | 13 "    | 13¾ "  | "            | "                                                      |         |        |         |         |                           |
| 378 | 1½ cc. attenuated germ .                               | 14 "    | 14 "   | Thin         | Dead from pneumonia                                    |         |        |         |         |                           |

| No.    | Dec. 21                  | Dec. 26                   | Dec. 21 | Feb. 2 | Feb. 12 | Mar. 6 | March 8               | Mar. 16 | Apr. 6         | Apr. 19 | Apr. 21 |
|--------|--------------------------|---------------------------|---------|--------|---------|--------|-----------------------|---------|----------------|---------|---------|
|        |                          |                           |         |        |         |        |                       |         |                |         |         |
| II.    | ¼ cc. of 61st generation | 1½ cc. of 61st generation | 13 oz.  | 14 oz. | 16 oz.  | .....  | 1½ cc. virulent germ. | 16 oz.  | Chloro- formed | 19 oz.  |         |
| III.   | " "                      | " "                       | 12 "    | 15½ "  | 14 "    | .....  | " "                   | 17 "    | 17 oz.         | 20 "    |         |
| IV.    | " "                      | " "                       | 15 "    | 16 "   | 17 "    | .....  | " "                   | 20 "    | 21 "           | 19 "    |         |
| V.     | " "                      | " "                       | 13 "    | 15 "   | 16 "    | .....  | " "                   | 17½ "   | 19 "           | 18 "    |         |
| VII.   | " "                      | " "                       | 14 "    | 13 "   | 14 "    | .....  | " "                   | 16 "    | 16½ "          | 18 "    |         |
| VIII.  | " "                      | " "                       | 15 "    | 15 "   | 16 "    | .....  | " "                   | 17 "    | 16 "           | 18 "    |         |
| IX.    | " "                      | " "                       | 12 "    | 13 "   | 11 "    | .....  | " "                   | 14 "    | 15 "           | 16 "    |         |
| X.     | (Check)                  | (Check)                   | .....   | 16 "   | 16 "    | 18 oz. | " "                   | 17½ "   | 14 "           | 12 "    |         |
| XI.    | (Check)                  | (Check)                   | .....   | 16 "   | 16 "    | 17 "   | " "                   | 17½ "   | Dead           | 19 oz.  | Dead    |
| XII.   | ½ cc. of 61st generation | 1½ cc. of 61st generation | 16 oz.  | 15 "   | 16 "    | .....  | " "                   | 18 "    | 19 oz.         | 19 "    |         |
| XIII.  | " "                      | " "                       | 14 "    | 15 "   | 16 "    | .....  | " "                   | 18 "    | 17½ "          | 16½ "   |         |
| XV.    | " "                      | " "                       | 15 "    | 16 "   | 18 "    | .....  | " "                   | 18 "    | 14 "           | 20 "    |         |
| XVI.   | " "                      | " "                       | 15 "    | 15 "   | 17 "    | .....  | " "                   | 18 "    | 18½ "          | 20 "    |         |
| XVII.  | " "                      | " "                       | 12 "    | 12 "   | 13 "    | .....  | " "                   | 15 "    | 15 "           | 14½ "   |         |
| XVIII. | " "                      | " "                       | 15 "    | 15 "   | 14 "    | .....  | " "                   | 16 "    | 17½ "          | 17½ "   |         |
| XIX.   | " "                      | " "                       | 14 "    | 13 "   | 13 "    | .....  | " "                   | 13 "    | 17½ "          | 12½ "   |         |
| XX.    | " "                      | " "                       | 15 "    | 18 "   | 14 "    | .....  | " "                   | 20 "    | 19 "           | 19 "    |         |

TABLE III.—EFFECT OF SERUM FROM HORSE INJECTED WITH ATTENUATED CULTURE ON TUBERCULOUS GUINEA-PIGS.

| No.         | Weight. | October 24. Amount of virulent culture and serum injected. |                             | Dates and amount of serum injected. |                           |                           |        |          |
|-------------|---------|------------------------------------------------------------|-----------------------------|-------------------------------------|---------------------------|---------------------------|--------|----------|
|             |         |                                                            |                             | November 16                         | November 17               | November 25               | Dec. 8 | April 19 |
| 434 (check) | 10 oz.  | $\frac{3}{4}$ cc. of vir. tuberc. cult.                    | .....                       | 10 oz.                              | 8 $\frac{1}{2}$ oz.       | 8 oz.                     | Dead   |          |
| 435         | 9 "     | " "                                                        | $\frac{1}{2}$ cc. of serum. | 9 oz. + $\frac{1}{2}$ cc.           | 8 oz. + $\frac{1}{2}$ cc. | 8 oz. + $\frac{1}{2}$ cc. | .....  | 20 oz.   |
| 436         | 11 "    | " "                                                        | " "                         | 10 " "                              | 9 " "                     | 7 " "                     | Dead   |          |
| 437         | 14 "    | " "                                                        | " "                         | 13 " "                              | 11 " "                    | 10 " "                    | .....  | 20 oz.   |
| 438         | 9 "     | " "                                                        | " "                         | 8 " "                               | 8 " "                     | 6 " "                     | Dead   |          |
| 439         | 8 "     | " "                                                        | " "                         | 8 " "                               | 7 " "                     | 5 " "                     | Dead   |          |

TABLE IV.—TEST OF DRY ANTITOXIC MATERIAL FROM SERUM FROM VACCINATED HORSE.

| No. of animal. | Weight.                 | Date.  | Material for inoculation.                                | Date.   | Weight. | March 8.            | Date.  | Weight. | Date.                                    |
|----------------|-------------------------|--------|----------------------------------------------------------|---------|---------|---------------------|--------|---------|------------------------------------------|
| 464            | Check 11 oz.            | Feb. 4 | $\frac{1}{8}$ cc. tuberc. virulent                       | Feb. 20 | 11 oz.  | .....               | Mar. 6 | 10 oz.  | March 8. Dead.                           |
| 476            | 12 "                    | " 4    | $\frac{1}{8}$ cc. tuberc. virulent + 0.208 gr. antitoxin | " 20    | 12 "    | .....               | " "    | 10 "    | " Dead; less disease than others.        |
| 478            | Check 8 $\frac{1}{2}$ " | " 4    | $\frac{1}{8}$ cc. tuberc. virulent                       | " 20    | 8 "     | Dead; tuberculosis. |        |         |                                          |
| 479            | " 8 "                   | " 20   | $\frac{1}{8}$ cc. tuberc. virulent                       | .....   | .....   | .....               | " "    | 7 "     | " Dead; generalized tuberculosis.        |
| 481            | " 10 "                  | " 20   | $\frac{1}{8}$ cc. tuberc. virulent                       | .....   | .....   | .....               | " "    | 9 "     | " Dead.                                  |
| 482            | 13 "                    | " 20   | $\frac{1}{8}$ cc. tuberc. virulent                       | .....   | .....   | .....               | " "    | 12 "    | April 7. Dead; less disease than others. |
| 484            | 12 "                    | " 20   | $\frac{1}{8}$ cc. tuberc. virulent + 0.008 gr. antitoxin | .....   | .....   | .....               | " "    | 12 "    | " Dead; less disease than others.        |







